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Research Section

Analysis of 200 food items for benzo[*a*]pyrene and estimation of its intake in an epidemiologic study[☆]

N. Kazerouni [a,c,*], R. Sinha [a], Che-Han Hsu [b,1], A. Greenberg [b,2], N. Rothman [a]

[a]*Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Executive Plaza South, Rm 7033, 6120 Executive Blvd, Rockville, MD 20892, USA*

[b]*Department of Environmental Sciences, Cook College, Rutgers University, New Brunswick, NJ 08903, USA*

[c]*Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814-4799, USA*

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Abstract

Animal studies have shown that dietary intake of benzo[*a*]pyrene (BaP), a polycyclic aromatic hydrocarbon (PAH), causes increased levels of tumors at several sites, particularly in the upper gastrointestinal tract. However, the role of dietary intake of BaP and cancer in humans is not clear. We created a BaP database of selected food products that could be linked to Food Frequency Questionnaires (FFQs) to estimate BaP intake. BaP levels were measured for each food line-item (composite samples) which consisted of a variety of foods in a FFQ. Composite sample parts were derived from the Second National Health and Nutrition Examination Survey (NHANES II) which represents the most common food items consumed by the general population. Meat samples were cooked by different techniques in controlled conditions, and by various restaurants and fast-food chains. Non-meat products were purchased from the major national supermarket chains. The quantities of BaP were measured using a thin-layer chromatography (TLC)/spectrofluorometer technique and were highly correlated with both BaP (radius = 0.99) and sum of carcinogenic PAH ($r = 0.98$) measured by HPLC technique. We linked our database to the results from a FFQ and estimated the daily BaP intake of various food items in 228 subjects in the Washington, DC metropolitan area. The highest levels of BaP (up to about 4 ng BaP/g of cooked meat) were found in grilled/barbecued very well done steaks and hamburgers and in grilled/barbecued well done chicken with skin. BaP concentrations were lower in meats that were grilled/barbecued to medium done and in all broiled or pan-fried meat samples regardless of doneness level. The BaP levels in non-meat items were generally low. However, certain cereals and greens (e.g. kale, collard greens) had levels up to 0.5 ng/g. In our population, the bread/cereal/grain, and grilled/barbecued meat, respectively, contributed 29 and 21 percent to the mean daily intake of BaP. This database may be helpful in initial attempts to assess dietary BaP exposures in studies of cancer etiology. Published by Elsevier Science Ltd.

Keywords: Polycyclic aromatic hydrocarbons; Benzo[*a*]pyrene; Meat; Pork; Chicken; Seafood; Vegetables; Fruit; Grains; Fat; Sweets; Pan-fry; Oven-broil; Grill; Barbecue; Carcinogenic; Food frequency questionnaire; Cancer

Abbreviations: BaP, benzo[*a*]pyrene; FFQ, Food Frequency Questionnaire; HCA, heterocyclic amine(s); PAH, polycyclic aromatic hydrocarbon(s); HHHQ, Health Habits and History Questionnaire; ng, nanogram; NHANES II, Second National Health and Nutrition Examination Survey; TLC, thin layer chromatography; THEES, Total Human Exposure to Environmental Substances.

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* Corresponding author. Tel.: +1301-594-7296; fax: +1301-496-1854.

E-mail address: kazeroun@exchange.nih.gov (N. Kazerouni).

[1] Current address: Department of Hospital and Health Care Administration, Chung-Tai Institute of Health Sciences and Technology, 11, Po-tze Lane, Takun, Taichung, 40605, Taiwan, ROC.

[2] Current address: Department of Chemistry, University of New Hampshire, Durham, NH 03824, USA.

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1. Introduction

Benzo[*a*]pyrene (BaP), a member of the polycyclic aromatic hydrocarbon (PAH) class, is one of the most potent PAH carcinogens in animal experiments (Howard and Fazio, 1980) and is embryotoxic and teratogenic in mice (IARC, 1983). Leukemia, gastric tumor and pulmonary adenoma or tumor developed in the strain of white Swiss mice that were fed BaP (Rigdon and Neal, 1966, 1969; Neal and Rigdon, 1967; Rigdon et al., 1967). A study by Weyand et al. (1995) showed an association between the ingestion of a diet containing BaP and forestomach tumors in A/J strain of mice. A more recent study by Culp et al. (1998) examined the histopathological sections of other sites such as small intestine, tongue and esophagus in female B6C3F1 mice. This study showed that BaP treatment in a 2-year feeding study resulted in an increased incidence of papillomas and/or carcinoma of forestomach, esophagus and tongue. Although the mechanism of BaP carcinogenicity in humans is not clear, a study by Autrup et al. (1982) investigated the BaP metabolism in cultured normal human bronchus, colon, duodenum and esophagus from the same patient. In that study, the highest total metabolism was found in bronchus and duodenum. The descending order for the 'BaP mean binding levels' was observed in bronchus, esophagus, duodenum and transverse colon. Because of species differences in carcinogenesis by BaP with respect to the site and type of tumor formation (Weyand and Bevan, 1987), BaP carcinogenicity in humans remains unclear.

BaP concentration is a good marker of carcinogenic PAH levels in environmental samples (Bjorseth, 1983; Butler et al., 1993). BaP is the most known and studied member of the PAH because it is one of the most potent PAH animal carcinogens (Howard and Fazio, 1980), and it is relatively easy to separate and analyze by longer wavelength fluorescence. BaP is present in a wide variety of food items (IARC, 1983). The Total Human Exposure to Environmental Substances (THEES) study had shown that people in the general population without substantial exposure to pollution and occupational exposure had a higher exposure to carcinogenic BaP by food ingestion than by inhalation (Butler et al., 1993).

PAH are found in grilled/barbecued meat, vegetables, oils, grains/cereals, fruits, smoked fish and seafood in concentrations as low as 0.001 ng/ per g (200 ppb) (Lijinsky and Ross, 1967; Lo and Sandi, 1978; Lintas et al., 1979; Howard and Fazio, 1980; Santodonato et al., 1981; Dennis et al., 1983; IARC, 1983; Larsson, 1986; Vaessen et al., 1988; De Vos et al., 1990; Greenberg et al., 1990; Lodovici et al., 1995). As there is no comprehensive PAH database, epidemiologic studies have not been able to estimate dietary PAH intake and investigate the risk of cancer associated with it. However, investigators have evaluated the relationship between the intake of several foods that may have high level of PAH (e.g. smoked or grilled/barbecued meats) and risk for cancer at several sites including stomach and esophagus (Soos, 1980; Ward et al., 1997), colorectal (Peters et al., 1989; O'Neill et al., 1990a,b; Schiffman and Felton, 1990; Gerhardson de Verdier et al., 1991; Lang et al., 1994; Muscat and Wynder, 1994; Sinha et al., 1999), pancreatic (Norell et al., 1986) and bladder cancer (Steineck et al., 1990). Because PAH are contained in wide variety of foods, it is necessary to directly estimate the dietary intake of BaP from all dietary sources to evaluate the relationship between dietary intake of BaP and risk of cancer.

Although there are extensive data on PAH in food, the data have not been collected in a way that could be incorporated in dietary questionnaires. Previous studies have looked at cooking methods in measuring BaP content in meats and poultry, but have not provided detailed BaP levels in meat cooked by a number of techniques and varying levels of doneness. In addition, previous studies have not reported BaP and/or total PAH concentrations in food in a way that could be linked to dietary questionnaires used in epidemiologic studies (Dennis et al., 1983; Vaessen et al., 1988; De Vos et al., 1990; Lodovici et al., 1995). The objectives of our study were to: (1) create a database of food items with BaP concentrations that could be linked to FFQs that have been used in epidemiologic studies; and (2) determine BaP intake using a FFQ in a disease-free population.

2. Materials and methods

2.1. Selection of food items

The first objective of our study was to create a database of food items with BaP concentrations that could be linked to a FFQ. We used a modified version of the Health Habits and History Questionnaire (HHHQ), a FFQ developed by the National Cancer Institute IMS Inc. and Block Dietary Data Systems (1994) to determine a variety of food items for which we could measure BaP concentrations. Each food line-item of this FFQ was a composite sample, for example, mixed greens: two parts mustard, one part turnip and one part collard greens of the house product (see Appendix A). Food line-item parts and their ratios were derived from the Second National Health and Nutrition Examination Survey (NHANES II) and the database developed by Gladys Block (Block et al., 1986).

We selected a variety of store-bought foods, including meat and non-meat items from two national supermarket chains that sold national and store brands, and purchased cooked meats from two fast-food or regular restaurants of a particular national chain within a 15-mile radius of Beltsville, Maryland. For each restaurant or fast food sample, four to six samples from the same store were purchased and pooled (Knize et al., 1995). Several samples were cooked in different sessions for each specific meat type, cooking method, and degree of doneness (length of cooking). A composite sample was made by pooling the cooked meat samples.

The meat products included cooked beef, chicken, and pork. We purchased three types of raw beef products: hamburger patties of freshly ground lean beef, steaks, and roasts, from a local supermarket. Hamburger patties and steaks were pan-fried, oven-broiled or grilled/barbecued, while roasts were cooked in an oven (Sinha et al., 1998a). For three hamburger doneness categories: medium, well done, and very well done, independent replicate samples were analyzed separately to assess within-category variation. Three types of chicken were purchased from a local supermarket: skinless, boneless chicken breasts; breasts with skin and bones; and whole chickens. Chicken breasts were either pan-fried, oven-broiled, or grilled/barbecued (Sinha et al., 1995). Whole chickens were roasted or stewed. Skinless, boneless chicken breasts were cooked to two levels of doneness: well done and very well done. Chicken breasts with bones and skin and whole chicken were cooked to only one degree of doneness, well done. The five pork products were purchased and cooked as previously (Sinha et al., 1998b) to three degrees of doneness: just until done, well done and very well done. Smoked or baked ribs and some sausages were purchased from national chain restaurants.

The store-bought non-meat items which included vegetables, fruits, cereals, grains, breads, sweets, dairy and fat products are listed in Appendix A and were fresh, frozen or cooked. For the subset of produce reported by previous studies to have high levels of PAH, we measured the PAH levels during various seasons. An average of these values for each sample is reported in Tables 5–8.

2.2. Measurement of BaP in food items

Samples of food items were saponified in alcoholic KOH, extracted into isooctane, cleaned up by column chromatography on florisil, and analyzed via TLC/fluorescence, as described previously (Greenberg et al., 1993), to detect the BaP content. The cleaned samples were first eluted with cyclohexane and then with benzene. The benzene fractions were used for PAH analysis. We used a plate-scanning spectrofluorometer (Perkin-Elmer MPF-44B) to monitor and quantitate BaP using 387 nm excitation and 428 nm emission wavelengths as previously described (Greenberg et al., 1990), 100 μl (0.1 ml) of each sample was applied as a spot on a 20% acetylated cellulose plate. Each sample was spotted in duplicate with five calibration solutions. The plate was developed in an ethanol/dichloromethane solution. The limit of detection for a single analysis of an aliquot of BaP was 0.005 ng/g or ppb weight.

Selected samples from each major food group were analyzed for BaP by both TLC and HPLC techniques. The samples were selected so that the distribution of food groups was about the same in both groups of analysis. As shown in Fig. 1, because the TLC and HPLC values for this pilot group of samples were highly correlated ($r = 0.998$; P value $= 0.0001$), we used the TLC technique to analyze the remaining samples.

To verify measurement reproducibility, pooled quality control samples with relatively low and high content of BaP were blindly interspersed throughout the analysis. These samples were made from hamburger pan-fried at

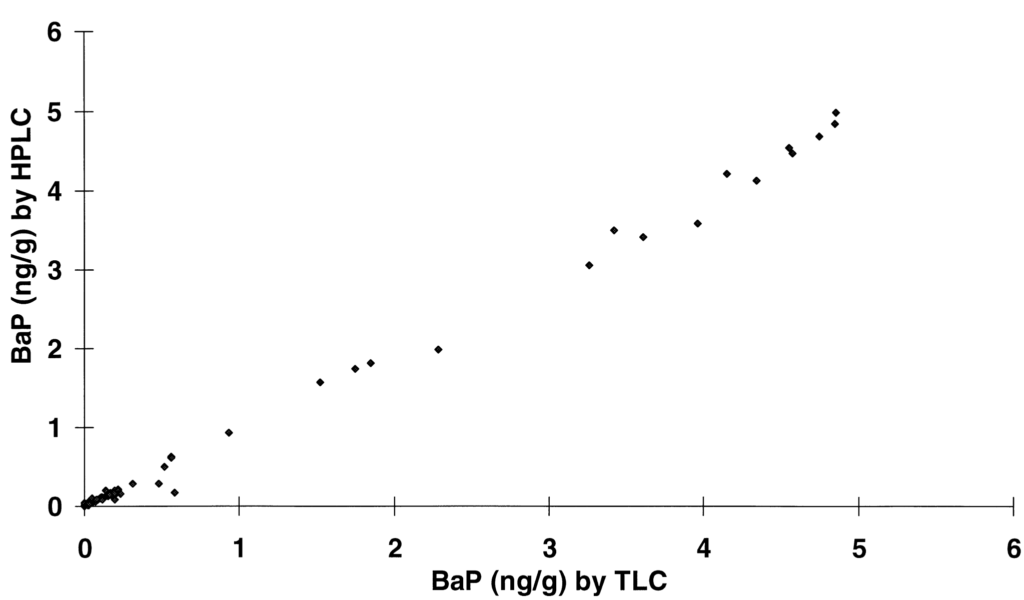

Fig. 1. Correlation of BaP in samples analysed by TLC and HPLC.

Table 1
Sample source and benzo[*a*]pyrene content in beef

Sample	Method	Source	Doneness	ng/g or ppb[a]	Medium portion size (g)	ng/medium portion size
Hamburger	Oven-broiled	Controlled cooking	Medium	0.01	85	0.8
			Well	0.01		0.8
			Very well	0.01		0.8
	Pan-fried	Controlled cooking	Medium	0.01	85	0.8
			Well	0.02		2.0
			Very well	0.01		0.8
	Grilled/barbecued	Controlled cooking	Medium	0.09	85	8.0
			Well	0.56		48
			Very well	1.52		129
Steak	Oven-broiled	Controlled cooking	Medium	0.01	112	1.0
			Well	0.01		1.0
			Very well	0.01		1.0
	Pan-fried		Medium	nd[b]		nd
			Well	0.01		1.0
			Very well	0.01		1.0
	Grilled/barbecued	Controlled cooking	Medium	4.15		465
			Well	4.75		532
			Very well	4.86		544
Salisbury steak	Baked	Controlled cooking	Well	0.01		1.0
Beef stewed		Controlled cooking		0.02	245	5.0
Roast	Baked	Controlled cooking	Medium	nd	112	nd
			Well	0.01		1.0
Beef gravy	Roast	Controlled cooking	Medium	0.01	30	0.3
			Well	0.01		0.3
Beef gravy, can		Grocery store		0.01		0.3
Brown gravy, package				0.02	30	0.6
Spaghetti sauce/meat		Controlled cooking		0.01	224	2.0
Vegetable/beef soup		Controlled cooking		0.02	184	4.0

[a] Limit of detection is 0.005 ng/g or ppb.

Table 2
Sample source and benzo[*a*]pyrene content in chicken or seafood

Sample	Method	Source	Doneness	ng/g or ppb[a]	Medium portion size (g)	ng/medium portion size
Chicken						
Chicken with bone & skin	Oven-broiled	Controlled cooking	Well	0.08	96	8.0
	Pan-fried		Well	0.12	102	12
	Grilled/barbecued		Well	4.57	96	439
Chicken/boneless	Oven-broiled	Controlled cooking	Well	0.12	96	11
			Very well	0.48	96	46
	Pan-fried		Well	0.10	102	10
			Very well	0.10	102	10
	Grilled/barbecued		Well	0.39	96	37
			Very well	0.40	96	38
Whole chicken	Stewed	Controlled cooking	Standard	0.01	96	1.0
	Roasted			0.01		1.0
Chicken gravy, pkg		Grocery store		0.04	30	1.0
Chicken gravy	Roasted	Controlled cooking	Standard	0.14	30	4.2
Chicken gravy, can		Grocery store		0.01	30	0.3
Chicken noodle soup		Grocery store		0.02	184	4.0
Seafood						
Crab		Controlled cooking		0.10	38	3.8
Perch fillet	Oven-broiled	Controlled cooking	Well	0.19	85	16
			Very well	0.24		20
	Pan-fried		Well	0.15		13
	Grilled/barbecued		Well	0.15		13
Smoked fish				0.10		8.5
Tuna, can/in oil				0.01	80	0.8
Tuna, can/water				0.01		0.8

[a] Limit of detection is 0.005 ng/g or ppb.

low temperature (containing low levels of BaP) or hamburger patties grilled at high temperature (containing high levels of BaP). Three samples of each low and high quality controls were analyzed together at the beginning, middle and end of the study to assess precision at those time periods. The average concentration of BaP found in the high temperature samples ($n=21$) and low temperature samples ($n=17$) were 2.6 ng/g (S.E.±0.2; coefficient of variation 0.40) and 0.13 ng/g (±0.01; 0.36), respectively. All quality assurance/control procedures, which included BaP recovery using Florisil pre-treatment/testing, and analysis of replicate food composites, were followed throughout the entire analysis process as described previously (Greenberg et al., 1990).

2.3. Comparison of BaP with other PAH in food items

To verify that BaP is a good marker for other PAH in food items, we calculated the Pearson correlation coefficients (r) between BaP values of a representative sample of food items and their carcinogenic PAH values or total PAH values. In a validation study, we selected samples from each major food groups to be analyzed for PAH, including BaP, by HPLC. Total PAH were the sum of naphthalene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene, and cyclopenta[*cd*]pyrene in our study food samples. The correlation coefficient between the total PAH values and the BaP value was 0.87 (P value 0.0001). Total carcinogenic PAH were the sum of benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, chrysene, and cyclopenta[*cd*]pyrene in the study food samples (IARC, 1983; Ross et al., 1995). The correlation coefficient between the carcinogenic PAH values and the BaP value measured by TLC was 0.98 (P value 0.0001). The lower correlation coefficient between the total PAH values and the BaP value can be an artifact of volatilization of lighter PAH members such as naphthalene, acenaphthene, fluorene, anthracene, and phenanthrene.

Table 3
Sample source and benzo[*a*]pyrene content in pork

Sample	Method	Source	Doneness	ng/g or ppb[a]	Medium portion size (g)	ng/medium portion size
Bacon	Oven-broiled	Controlled cooking	Well	nd[b]	16	nd
			Very well	nd		nd
	Microwave		Well	0.01		0.2
			Very well	0.02		0.3
	Pan-fried		Just	0.01		0.02
			Well	0.2		0.3
Bacon fat	Pan-fried	Controlled cooking	Well	nd	10	nd
			Very well	nd		nd
Salami				0.01	56	0.6
Bologna				0.02	56	1.0
Ham slice	Oven-broiled	Controlled cooking	Well	nd	28	nd
			Very well	0.01		0.3
	Pan-fried		Well	0.01		0.3
			Very well			
Smoked ham				0.13	56	7.3
Ham/lunch meat				0.01	56	0.6
Pork chops	Oven-broiled	Controlled cooking	Well	0.01	112	1.0
			Very well			
	Pan-fried		Well	0.01	112	1.0
			Very well			
Hot dogs	Oven-broiled	Controlled cooking	Well	0.01	88	0.9
			Very well	0.01		0.9
	Pan-fried		Well	0.02		2.0
			Very well	0.03		3.0
	Grilled/barbecued		Well	0.01		0.9
			Very well	0.05		4.0
Sausage links	Pan-fried	Controlled cooking	Well	0.02	54	1.0
	Package directions	Grocery store	Very well	0.01		0.05
			Just/well	nd		nd
Sausage patties	Pan-fried	Controlled cooking	Well	0.03	54	2.0
			Very well	0.02		1.0

[a] Limit of detection is 0.005 ng/g or ppb.
[b] nd, not detectable.

Table 4
Sample source and benzo[*a*]pyrene content in restaurant/fast-food meat items

Sample	Method	Source	Doneness	ng/g or ppb[a]	Medium portion size (g)	ng/medium portion size
Hamburger	Pan-fried	Restaurants 1 and 2	Medium	0.15	176	26
			Well	0.13		23
			Very well	0.12		21
		Fast-food A		0.02	112	2.0
		Fast-food B		0.07		8.0
		Fast-food C		0.02		2.0
	Grilled/barbecued	Restaurants 3 and 4	Medium	0.73	176	128
			Well	1.23		215
			Very well	1.45		255
		Fast-food D		1.75		196
Steak	Pan-fried	Restaurants 1 and 2	Medium	0.12		13
			Well	0.12		13
			Very well	0.12		13
	Grilled/barbecued	Restaurants 3 and 4	Medium	0.80		90
			Well	0.81		91
			Very well	1.62		181
Ribs (pork)	Smoked	Restaurants 1 and 4	Usual	0.16		
			Well	0.15		
	Baked	Restaurant 3	Usual	1.64		
			Well	0.93		
Sausage (pork)	Grilled/barbecued	Vendor A	Well	0.05	54	3.0
		Vendor B	Well	0.05		3.0
		Vendor D	Well	0.11		5.9
Pepperoni pizza		Vendor A and B		0.23	121	28
Chicken breast	Deep-fried	Fast food E		0.06	148	9.0
		Fast food F		0.01		1.0
		Fast food C		0.14		21
		Fast food A		0.08		12
Fish sandwich	Deep-fried	Fast food D		0.07	112	8.0
		Fast food A		0.02		2.0
		Fast food C		0.03		3.0

[a] Limit of detection is 0.005 ng/g or ppb.

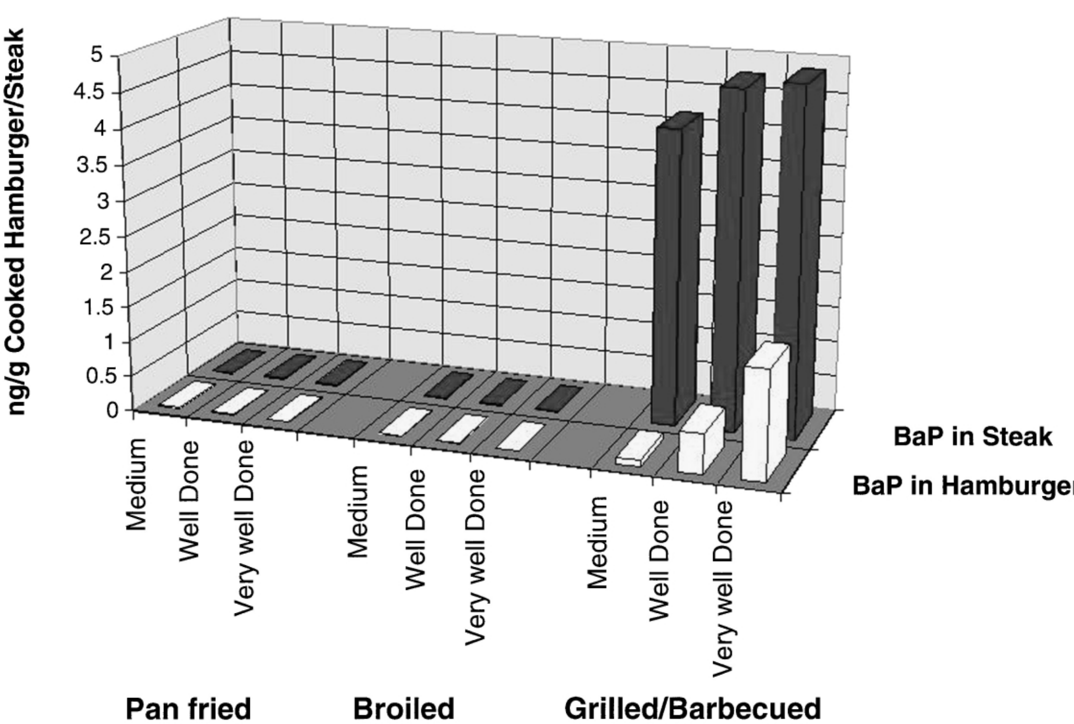

Fig. 2. BaP content of hamburger and steak cooked by different methods to varying degrees of doneness.

2.4. BaP content of foods

We present our BaP data in two ways: (1) by concentration of BaP in nanograms per gram of food item, and (2) by amount of BaP per ng in a "medium" portion size as used in HHHQ. A "medium" portion size is a standard amount consumed by subjects for that particular food item, for example, a "medium" portion size for butter is 10 g while for hamburger is 85 g. We multiplied the concentrations of BaP (ng/g) in each food line-item by its designated "medium" portion size used in the HHHQ to obtain the amount of BaP.

Table 5
Levels of benzo[*a*]pyrene in dairy products, fat products and beverages

Food type	ng/g or ppb[a]	Portion size (g)	ng/medium portion size
Butter	nd[b]	10	nd
Cheese, American	nd	56	nd
Corn oil	nd	15	nd
Cottage cheese	0.07	113	8.0
Cream	0.16	15	2.4
Diamond almonds	nd	18	nd
Diamond walnuts	0.03	18	0.5
Eggs	0.03	100	3.0
Margarine	0.12	14	1.7
Mayonnaise	0.03	32	1.0
Milk, whole	0.02	244	5.0
Mixed nuts	nd	18	nd
Non-dairy cream/liquid	0.12	15	1.8
Non-dairy cream/powder	nd	3	nd
Peanut butter	nd	18	nd
Peanuts	0.01	18	0.2
Cashews	0.02	18	0.4
Vegetable oil	0.02	15	0.3
Yogurt, flavored/frozen	0.18	227	41

[a] Limit of detection is 0.005 ng/g or ppb.
[b] nd, non detectable.

Table 6
Levels of benzo[*a*]pyrene in breads, salty snacks, cereals and grains

Food type	ng/g or ppb[a]	Portion size (g)	ng/medium portion size
Biscuits	0.13	85	11
Blueberry muffin	0.03	85	3.0
Whole oat cereal	0.08	42	3.0
Corn chips	0.06	28	2.0
Corn flakes	0.15	28	4.2
Corn muffins	0.09	85	8.0
Cream of wheat	0.31	160	50
Fast-food biscuit	0.08	85	7.0
Low fat granola cereals with raisin	0.30	28	8.4
Grits	0.17	160	27
Fortified cereal	0.02	28	0.6
Unpopped popcorn	0.51	28	14
100% bran	0.03	28	0.8
Oatmeal	0.18	160	29
Popped popcorn	0.56	28	16
Natural bran	0.02	36	0.7
Potato chips	0.04	28	1.0
Pretzels	0.37	28	10
Rice	0.12	131	16
Rice krispies	0.11	28	3.1
Shredded wheat	0.25	28	7.0
Spaghetti	0.18	125	22
White bread	0.10	50	5.0
Fast-food french fry	0.22	102	22

[a] Limit of detection is 0.005 ng/g or ppb.

2.5. Determination of intake of BaP in a disease-free population

Our second objective was to determine the intake of BaP in a control population. We used the FFQ completed by male/female controls who participated in a case–control study of colorectal adenomas at the National Naval Medical Center in Bethesda, Maryland (Sinha et al., 1999). This questionnaire consisted of details on frequency of consumption and portion sizes (small, medium or large) of different food items. Responses from the FFQ were used to estimate consumption (in g/day) of the different food line-items using frequency and portion size. BaP levels were derived by multiplying grams of the food line-items by their BaP content. The Bap content was then totaled across all food line-items in the diet to estimate intake for individual study subjects.

3. Results

The BaP concentrations of the various food items are shown in Tables 1–8 as ng/g as well as the amount in a "medium" portion size. Items with the highest concentration of BaP were very well done grilled/barbecued steak, well done grilled/barbecued chicken with skin, very well done grilled/barbecued hamburger, collard greens and kale, pumpkin pie, pretzels, bran and granola cereal, cooked cereal, margarine and french fries. The descending order for BaP concentration among the

Table 7
Levels of benzo[*a*]pyrene in sweets and desserts

Food type	ng/g or ppb[a]	Portion size (g)	ng/medium portion size
Assorted cakes	0.11	42	4.6
Assorted cookies	0.01	42	0.4
Cake with icing	0.02	42	0.8
Chocolate candy	0.18	30	5.5
Doughnuts	0.03	42	1.0
Granola bars	0.09	28	2.0
Grape jelly	0.01	15	0.1
Non-chocolate candy	0.23	9	2.1
Other pies/apple and cherry	0.11	135	15
Pumpkin pie	0.47	135	63
Sugar	0.15	8	1.2

[a] Limit of detection is 0.005 ng/g or ppb.

top ten items with the highest concentrations of BaP in a ''medium'' portion size (ng/medium portion size) was somewhat different than the order for items with highest concentrations of BaP in ng/g. This order for BaP concentration among the former items was very well done grilled/barbecued steak, well done grilled/barbecued chicken with skin, very well done grilled/barbecued hamburger, pumpkin pie, very well done broiled skinless chicken, collard and kale, very well done grilled/barbecued skinless chicken, pepperoni pizza, orange/tangerine and tomato.

Table 8
Levels of benzo[*a*]pyrene in fruits and vegetables

Food type	ng/g or ppb[a]	Portion size (g)	ng/medium portion size
Apples/apple sauce/pears	0.10	138	14
Banana	0.16	119	19
Broccoli	0.17	85	14
Canned corn	0.17	83	14
Canned green beans	0.14	67	9.4
Canned peas	0.09	83	7.0
Cantaloupe	0.01	136	1.0
Carrots	0.15	75	11
Cauliflower	0.12	90	11
Cole slaw	0.02	60	1.0
Collard greens	0.48	90	43
Strawberry, frozen	0.01	120	1.0
Grapefruit	0.02	185	4.0
Kale, frozen	0.15	90	13
Kale	0.47	90	42
Mixed greens, frozen	0.14	90	13
Mustard greens	0.13	50	6.5
Oranges	0.16	145	23
Peaches/apricots in syrup	0.17	100	17
Spinach, frozen	0.12	41	4.9
Spinach, fresh and frozen	0.10	41	4.1
Winter squash	0.18	123	22
Sweet potato, fresh and canned	0.17	100	17
Tomato	0.19	121	23
Turnip greens	0.10	90	9.0
White potato	0.17	93	16

[a] Limit of detection is 0.005 ng/g or ppb.

As seen in Tables 1–4, there was a striking variation in BaP concentration in meat items. The highest BaP concentration found among the meat samples was 4.86 ng/g in a very well done grilled/barbecued steak, whereas the very well done grilled/barbecued hamburger contained 1.52 ng/g of BaP. In contrast, very well done broiled, pan-fried hamburgers and steak contained low levels of BaP (0.01 ng/g). As shown in Fig. 2, comparing different cooking methods, grilled/barbecued steak samples had much higher content of BaP than hamburgers cooked with the same method. In addition, there was a striking difference in the BaP concentrations for different doneness levels between grilled samples of hamburger and steak. The BaP concentration decreased from 4.57 ng/g for well done grilled/barbecued chicken with skin to 0.39 ng/g for skinless chicken. However, the oven-broiled chicken (Table 2) showed an average concentration of 0.1 ng/g of BaP. In perch fillets, the BaP concentration did not differ by cooking method or doneness levels and showed a mean level of 0.17 ppb. Among classes of meat, pork had low levels of BaP except for baked ribs (Tables 3 and 4). In Table 4, the restaurant meat items showed a similar trend for BaP concentrations as in meat samples cooked in controlled conditions. However, among restaurant meat items, there was no striking difference in BaP concentrations between grilled/barbecued hamburger and grilled/barbecued steak samples cooked at different doneness levels.

For most non-meat products (Tables 5–8), BaP levels were low. Among the dairy and fat products in Table 5, flavored/frozen yogurt had the highest BaP content per gram. In breads, salty snacks, cereals and grains (Table 6), popcorn had the highest level of BaP at 0.56 ppb. Among the sweets and desserts items (Table 7),

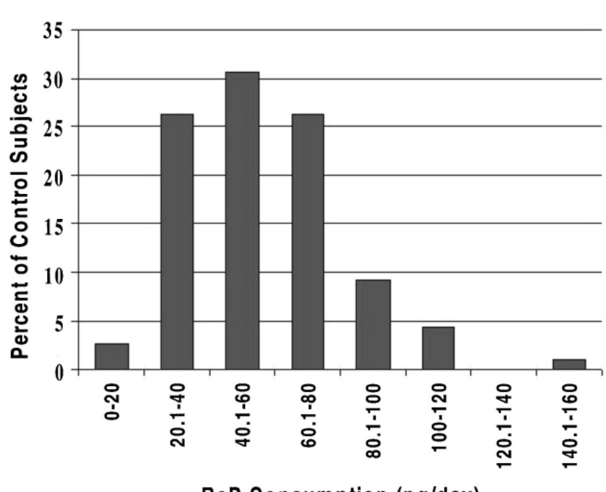

Fig. 3. Distribution of daily BaP intake in a control group of 228 subjects.

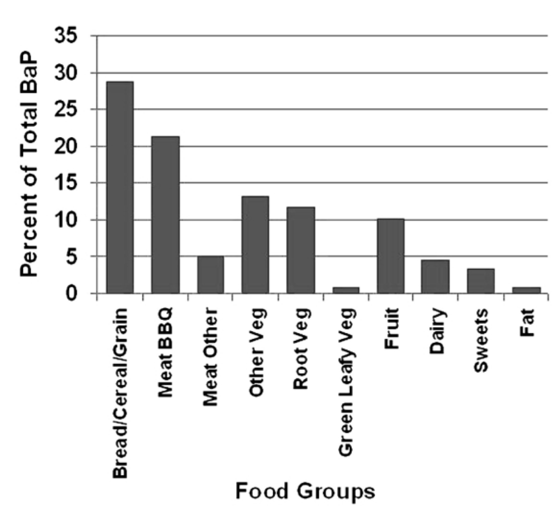

Fig. 4. Percent of total daily BaP intake in a control group of 228 subjects.

low levels of BaP were detected in all samples. In the analysis of vegetables and fruits (Table 8), the highest level per gram was found in collards and kale with levels of 0.48 and 0.47 ng/g or ppb, respectively.

Fig. 3 presents the distribution of daily BaP intake among our subjects. About 31% of the subjects had a BaP intake within the range of 40.1 to 60 ng per day. Fig. 4 presents the percent contribution of various food groups to the mean daily intake of BaP among the subjects. The bread/cereal/grain, meat, vegetables and fruit groups are the highest food group contributors to BaP intake, whereas the fat, sweet and dairy food groups make the smallest contribution.

4. Discussion

Our data showed that grilled/barbecued steak, well done grilled/barbecued chicken with skin, and very well done grilled/barbecued hamburger had the highest concentrations of BaP, but the BaP content in grilled/barbecued steak samples were much higher than grilled/barbecued hamburger samples. Grilled/barbecued meat contributed 21% to the total mean daily BaP intake in our subjects. Although the bread/cereal/grain food group contributed 29% to the total BaP intake in the subjects, the individual items in this food group had low levels of BaP. The top five items with the highest percent of daily BaP intake in our subjects were medium done grilled/barbecued steak, white potato, rice, tomatoes/tomato juice and cooked cereal. While collard greens, kale leafy vegetables and pumpkin pie were among the top ten items with the highest concentrations of BaP and amount of BaP in a "medium" portion size, they were not eaten frequently enough to make large contributions to the diet of study subjects. In general, BaP levels in our study food items were consistent with previous data (Dennis et al., 1983; Lawrence and Weber, 1984a,b; Vaessen et al., 1984; Larsson, 1986; De Vos et al., 1990; Lodovici et al., 1995; Knize et al., 1999).

In meats, BaP formation is largely affected by the cooking method (Larsson, 1983; Creighton et al., 1992) and doneness level (Davies and Wilmshurst, 1960; Masuda et al., 1967). In our study, grilled/barbecued meats contained higher levels of BaP compared to broiled or pan-fried meats, consistent with previous studies (Larsson, 1983; Lijinsky, 1991; Lodovici et al., 1995; Knize et al., 1999). Because PAH form on or near the surface of meats rather than in the interior, foods cooked without being exposed to smoke do not show significant levels of PAH. PAH are generated through pyrolysis during the charbroiling of meat products in which fat from the meat falls onto hot coals (Lijinsky, 1991). The incomplete combustion of carbon and hydrogen in fat sends up a column of smoke that coats the food with carcinogenic PAH (Lijinsky and Ross, 1967; Fazio et al., 1973; Larsson et al., 1983). In addition, the concentration of BaP in grilled/barbecued meats is affected by the length of cooking time (Knize et al., 1985; Larsson, 1986) or the level of doneness. In our study, higher levels of BaP were formed in very well and well done grilled/barbecued meat items, as compared to medium. Meats cooked to higher levels of doneness are in contact with heat and smoke for a greater length of time.

It is interesting to note that heterocyclic amines (HCA) — which are animal carcinogens — are formed by pan frying and broiling (Sugimura et al., 1988; Knize et al., 1995). Our PAH database along with a previously created HCA database (Sinha et al., 1998a,b), both of which used the same meat samples, may be helpful in determining how these compounds work independently from each other in studies of cancer etiology.

It has been well-established that there are two major sources of PAH formation in foods. According to Lijinsky (1991), PAH are generated through pyrolysis during charbroiling of meat products. The other major source of contamination of foodstuffs is by contact with either petroleum products or coal tar products. Because of the geochemical processes and atmospheric deposition of air pollution particulate on the crops, it would be possible to generate these naturally occurring PAH in foods (Dunn, 1982). The presence of PAH in the environment lead to the presence of PAH in foodstuffs.

The higher BaP content in green leafy vegetables such as kale and collard greens, compared with other vegetables in our study, could be explained by their greater contact surface to the ambient air during growth and their late harvest during the winter months. Through atmospheric deposition, PAH can contaminate the surfaces of leaf vegetables and growing fruits. Because air emission regulations are now more strict and the environmental conditions are different than previously, we detected lower levels of BaP in vegetables and fruits than what was reported before the 1970s. However, in a recent Italian study, all vegetables except tomato, exposed to polluted air, contained high levels of carcinogenic PAH (Lodovici et al., 1995). In addition, in a selection of reported BaP concentrations in food by Greenberg et al. (1990), the level of BaP in kale was in a range of 12.6–48.1 ng/g. It seems that the PAH levels in uncooked foods largely depend on the origin of the food and can be subject to regional variations.

The occurrence of BaP in seafood may be attributed to aquatic pollution as opposed to cooking methods, unless they were smoked (Howard and Fazio, 1980; Larsson, 1982; Lawrence and Weber, 1984b; Yabiky et al., 1993). Fish, shell fish, and other seafood may be contaminated by petroleum pollution indirectly through water contact in the marine environment. However, the very well-done broiled perch fillet showed higher levels of BaP than the well-done grilled/ barbecued sample.

This also indicates the importance of the level of doneness in the formation of BaP.

We found that the bread/cereal/grain group, followed by the grilled/barbecued meat group, contributed the most to the percent of total daily BaP intake in our control subjects. In contrast, uncooked fat, sweets and dairy food groups contributed the least. Considering the BaP concentration for individual cereal, fruit and vegetable food group items, the large contribution of each food group is attributed to its high consumption in our population. In terms of percent contribution to total BaP intake, our result is consistent with a study by Lodovici et al. (1995) for bread/cereal/grain, vegetables, fruit, sweet and dairy groups, but not for grilled/barbecued meat and dairy groups. The percent contribution to total BaP intake in our study, however, is inconsistent with other studies for the contribution of fats/oil (Larsson, 1986), and sweets (De Vos et al., 1990). In contrast with these studies, we found a lower percent contribution to BaP intake by these two food groups.

This study has a number of strengths. The primary strength of this study is that it is the first study of its kind to report BaP concentration in food items in a way that can be linked to a FFQ, which can in turn be used in epidemiologic studies to assess cancer risk due to dietary PAH exposures. Additionally, this database can be used along with the HCA database to assess dietary exposures from PAH and HCA — independently from each other — in epidemiologic studies. Certain limitations must be taken into account when interpreting these results. First, we did not have multiple samples to rule out possible variations in BaP concentration in each study sample. However, for a subset of samples, repeat measurements of BaP were performed seasonally throughout the year. These repeat measurements are included in the average values reported, accounting for seasonal effects on BaP concentration. Secondly, our database only includes the most common food items consumed in the American diet, and therefore applicable to populations with similar dietary patterns. Furthermore, although a national database (NHANES II) was used to determine the parts of composite samples for each cell items in a FFQ, the foods collected were limited to one region in Maryland, which included house brands as well as national brands; as such, the broad applicability of our database for foods is uncertain.

In conclusion, we created an initial database that can be linked to FFQs to assess dietary exposures of BaP in epidemiologic studies. This database is unique in that analyzed samples were derived from relevant food line-items in a FFQ. The parts of each sample were determined from a national survey (NHANES II) that reflected the most commonly consumed food items in the American diet. Although additional data is required to test seasonal and geographic variations for wide applicability, this database may be helpful in preliminary assessments of dietary PAH exposures in epidemiologic studies.

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Appendix A

Line item	Sources of composite sample
Biscuits	1 part fresh & 2 parts refrigerated house brand, 3 parts national brand
Fast-food biscuits	National fast-food restaurants
White bread	1 part light national brand, 1 part regular national brand, 5 parts house brand
Cold cereals, such as corn flakes, rice krispies	Corn flakes: 5 parts national brand, 1 part house brand Rice krispies: 5 parts national brand, 1 part house brand National brand
Cooked cereals	Oatmeal: National brand Grits: 1 part regular national brand, 1 part original flavor national brand Cream of wheat: 1 part regular national brand, 1 part original flavor national brand
High fiber, bran or granola cereals, shredded wheat	Natural bran, 100% bran, shredded wheat: National brand Low fat granola with raisins: 1:1 part different national brands

Line item	Sources of composite sample
Highly fortified cereals	National brand
Corn chips	5 parts national brands, 1 part house brand
Blueberry muffin	2 parts frozen national, 1 part fresh house, 1 part donut from a national chain
Corn muffins	1 part fresh house brand, 1 part donut from a national chain
Popped popcorn	2 parts national brand with cheddar, 1 part plain national brand, 1 part plain house brand
Unpopped popcorn	2 parts national brand, 1 part house brand
Potato chips	2 parts house brand, 1:10 part different national brands
Pretzel	2 parts house brand, 1:10 part different national brands
Rice	4 parts house brand, 1 part national brand
Spaghetti	5:1 part different house brands
Assorted cakes	1:1 part different national brands
Cake with icing	1 part house brand, 1 part national brand
Chocolate candy	2:1:1:1:1 part different national brands
Non-chocolate candy	1:1:1:1 part different national brands
Assorted cookies	3:2 part different national brands
Doughnuts	1 part house brand powdered cake, 5 parts donuts from a national chain
Granola bars (all flavors of variety)	1:1 part different national brands
Grape jelly	1 part national brand, 1 part house brand
Payday	National brand
Other pies/apple & cherry	1 part house cherry, 1 part house apple, 2:4 parts different national brands
Pumpkin pie	2 parts frozen national brand, 1 part fresh house brand
Sugar	1 part national brand, 1 part house brand
Apple/apple sauce/pears	5 parts fresh house apples, 2 parts house applesauce, 1 part national brand applesauce, 2 parts fresh house pears
Banana	1:1 part different national brands
Broccoli	2 parts fresh national product, 1 part frozen house product
Cantaloupe	1:1 different house brands
Carrots	2 parts fresh & 1 part frozen national products, 1 part frozen house product
Cauliflower	2 parts fresh & 1 part frozen national products
Coleslaw	1:1 different national brands
Collard greens	5 parts fresh & 3 parts frozen from a national chain store
Canned corn	1 part house brand, 1:1 part different national brands
Grapefruit	1:1 part different house brands
Canned green beans	1 part national brand, 1 part house brand
Mixed greens, frozen	2 parts mustard, 1 part turnip & 1 part collard greens of the house product
Mustard greens	5 parts raw & 3 parts frozen from a national chain store
Turnip greens	5 parts fresh & 3 parts frozen from a national chain store
Kale, frozen	1 part chopped house, 1 part leaf house product
Kale	5 parts fresh & 3 parts frozen from a national chain store
Oranges	1 part fresh national brand, 2 parts fresh house juice
Canned peas	1 part house brand, 1:1 different national brands
Peaches/apricots in heavy syrup	6 parts house peaches, 4 parts national brand peaches, 3 parts house apricots
White potatoes	1:1 part fresh different house products
Spinach, frozen	2 parts chopped house, 1 part leaf house product
Spinach	5 parts fresh national product, 2 parts frozen chopped house, 1 part frozen leaf house product
Winter squash	1:1 part frozen cooked different house products

Line item	Sources of composite sample
Frozen strawberry	1:1 part different house brands
Sweet potatoes	1 part fresh baked national product, 2 parts canned national brand
Tomatoes	1 part fresh house tomatoes, 3 parts canned house brand, 1 part canned national brand
Diamond almonds (lightly salted)	National brand
Butter	1.25 part national brand, 1 part house brand
Cashews	National brand
Cheese, American	2 parts house brand, 3 parts national brand
Cottage cheese	1 part house brand, 1 part national brand
Cream	1 part house brand, 1 part national brand
Non-dairy cream/liquid	2 parts national brand, 1 part house farm rich brand
Non-dairy cream/powder	1 part house brand, 1:1 part different national brands
Diamond walnuts	National brand
Eggs (large fresh)	1:1 part different house brands
Fast-food french fry	6:2:1:1 part different national fast food restaurants
Margarine	2:2:1 part different national brands, 1 part house brand
Mayonnaise	2:1 part different national brands, 1 part house brand
Milk, whole	House brand
Mixed nuts (no peanuts)	National brand
Corn oil	4 parts different (3:1) national brands, 1 part house brand
Vegetable oil	4 parts different (2:2) national brands, 1 part house brand
Peanut butter	1:1 part different national brands, 1 part house brand
Peanuts	1 part house brand, 2 parts national brands
Flavored/frozen yogurt	4 parts vanilla, 2 parts strawberry & 2 parts blueberry of the national brands; 1 part strawberry & 1 part blueberry of the house brand

References

Autrup, H., Grafstrom, R.C., Brugh, M., Lechner, J.F., Haugen, A., Trump, B.F., Harris, C.C., 1982. Comparison of benzo(a)pyrene metabolism in bronchus, esophagus, colon, and duodenum from the same individual. Cancer Research 42, 934–938.

Bjorseth, A., 1983. Handbook of Polycyclic Aromatic Hydrocarbons. Marcel Dekker, New York.

Block, G., Hartman, A.M., Carroll, M.D., Gannon, J., Gardner, L., 1986. A data-based approach to diet questionnaire design and testing. American Journal of Epidemiology 124, 453–469.

Butler, J.P., Post, G.B., Lioy, P.J., Waldman, J.M., Greenberg, A., 1993. Assessment of carcinogenic risk from personal exposure to benzo(a)pyrene in the total human environmental exposure study (THEES). Journal of the Air and Waste Management Association 43, 970–977.

Creighton, P.J., Greenberg, A., Lioy, P.J., 1992. The effect of cooking methodology on benzo[*a*]pyrene exposure from "home-cooked" bacon and hamburgers and "fast-food chain" hamburgers. Journal of Exposure Analysis and Environmental Epidemiology, Suppl. 2 2, 27–43.

Culp, S.J., Gaylor, D.W., Sheldon, W.G., Goldstein, L.S., Beland, F.A., 1998. A comparison of the tumors induced by coal tar and benzo[*a*]pyrene in a 2-year bioassay. Carcinogenesis 19, 117–124.

Dennis, M.J., Massey, R.C., McWeeny, D.J., Knowels, M.E., 1983. Analysis of polycyclic aromatic hydrocarbons in UK total diets. Food and Chemical Toxicology 21, 569–574.

De Vos, R.H., Van Dokkum, W., Schouten, A., De Jong-Berkhout, P., 1990. Polycyclic aromatic hydrocarbons in dutch total diet samples (1984–1986). Food and Chemical Toxicology 28, 263–268.

Dunn, B.P., 1982. Polycyclic aromatic hydrocarbons. In: Stich, H.F. (Ed.), Carcinogens and Mutagens in the Environment, Vol. I. CRC Press, Boca Raton, FL.

Fazio, T., White, R.H., Howard, J.W., 1973. Collaborative study of the multicomponent method for PAH in foods. Journal of the Association of Official Analytical Chemists 56, 68–70.

Gerhardsson de Verdier, M., Hagman, U., Peters, R.K., Steinech, G., Overvik, E., 1991. Meat, cooking methods and colorectal cancer: a case referent study in Stockholm. International Journal of Cancer 49, 520–525.

Greenberg, A., Hsu, C-H., Rothman, N., Strickland, P.T., 1993. PAH profiles of charbroiled hamburgers: Pyrene/B[*a*]P ratios and presence of reactive PAH. Polycyclic Aromatic Compounds 3, 101–110.

Greenberg, A., Luo, S., Hsu, C-H., Creighton, P., Waldman, J., Lioy, P.J., 1990. Benzo[*a*]pyrene in composite prepared meals: Results from the THEES (Total Human Exposure to Environmental Substances) study. Polycyclic Aromatic Compounds 1, 221–231.

Howard, J.W., Fazio, T., 1980. Review of polycyclic aromatic hydrocarbons in foods. Analytical methodology and reported findings of polycyclic aromatic hydrocarbons in foods. Journal of the Association of Official Analytical Chemists 63, 1077.

IARC, 1983. IARC Monographs on the Evaluation of Carcinogenic Risk of the Chemicals to Humans. Vol. 32. Polynuclear Aromatic Compounds, Part 1: Chemical, Environmental and Experimental Data. pp. 46–48, 211–373. International Agency for Research on Cancer, Lyon.

Knize, M.G., Andersen, B.D., Healy, S.K., Shen, N.H., Lewis, P.R., Bjeldanes, L.F., Hatch, F.T., Felton, J.S., 1985. Effect of temperature, patty thickness and fat content on the production of mutagens in fried ground beef. Food and Chemical Toxicology 23, 1035–1040.

Knize, M.G., Sinha, R., Rothman, N., Brown, E.D., Salmon, C.P., Levander, O.A., Cunningham, P.L., Felton, J.S., 1995. Heterocyclic amine content in fast-food meat products. Food and Chemical Toxicology 33, 545–551.

Knize, M.G., Salmon, C.P., Pais, P., Felton, J.S., 1999. Food heating and the formation of heterocyclic aromatic amine and polycyclic aromatic hydrocarbon mutagens/carcinogens. Advances in Experimental Medicine and Biology 459, 179–193.

Lang, N.P., Butler, M.A., Massengill, J., Lawson, M., Stotts, R.C., Hauser-Jensen, M., Kadulbar, F.F., 1994. Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increases the risk for colorectal cancer or polyps. Cancer Epidemiology Biomarkers & Prevention 3, 675–682.

Larsson, B.K., 1982. Polycyclic aromatic hydrocarbons in smoked fish. Zeitschrift für Lebensmitteluntersuchung und Forschung 174, 101–107.

Larsson, B.K., 1983. Polycyclic aromatic hydrocarbons. Internal report 1983-10-19. National Food Administration, Uppsala, Sweden.

Larsson, B.K., Sahlberg, G.P., Eriksson, A.T., Busk, L.A., 1983. Polycyclic aromatic hydrocarbons in grilled food. Journal of Agricultural and Food Chemistry 31, 867–873.

Larsson, B.K., 1986. Formation of polycyclic aromatic hydrocarbons during the smoking and grilling of food. Genetic Toxicology of the Diet 169–180.

Lawrence, J.F., Weber, D.F., 1984a. Determination of polycyclic aromatic hydrocarbons in Canadian samples of processed vegetable and dairy products by liquid chromatography with fluorescence detection. Journal of Agricultural and Food Chemistry 32, 794–797.

Lawrence, J.F., Weber, D.F., 1984b. Determination of polycyclic aromatic hydrocarbons in some Canadian commercial fish, shellfish, and meat products by liquid chromatography with confirmation by capillary gas chromatography-mass spectrometry. Journal of Agricultural and Food Chemistry 32, 789–793.

Lintas, C., De Matthaeis, M.C., Merli, F., 1979. Determination of benzo[*a*]pyrene in smoked, cooked and toasted food products. Food and Cosmetics Toxicology 17, 325–328.

Lijinsky, W., Ross, A.E., 1967. Production of carcinogenic polynuclear hydrocarbons in cooking of food. Food and Cosmetics Toxicology 5, 343–347.

Lijinsky, W., 1991. The formation and occurrence of polynuclear aromatic hydrocarbons associated with food. Mutation Research 259, 252–261.

Lo, M.-T., Sandi, E., 1978. Polycyclic aromatic hydrocarbons in (polynuclears) in foods. In: Gunther, F.A. (Ed.), Residue Review. Vol. 69. Springer-Verlag, New York, pp. 35–86.

Lodovici, M., Dolara, P., Casalini, C., Ciappellano, S., Testolin, G., 1995. Polycyclic aromatic hydrocarbon contamination in the Italian diet. Food Additives and Contaminants 12, 703–713.

Muscat, J.E., Wynder, E.L., 1994. The consumption of well-done red meat and the risk of colorectal cancer. American Journal of Public Health 84, 856–858.

National Cancer Institute IMS Inc. Block Dietary Data Systems., 1994. DIETSYS user's guide. National Cancer Institute, Bethesda, MD.

Neal, J., Rigdon, R.H., 1967. Gastric tumors in mice fed benzo(a)-pyrene: a quantitative study. Texas Reports on Biology and Medicine 25, 553–557.

Norell, A.E., Ahlbom, A., Erwald, R., Jacobson, G., Linberg-Navier, I., Olin, R., Tornberg, B., Wiechel, K.L., 1986. Diet and pancreatic cancer: a case-control study. American Journal of Epidemiology 124, 894–902.

O'Neill, I.K., Bingham, S., Povey, A.C., Brouet, I., Bereziat, J.C., 1990a. Modulating effects on human diets of dietary fiber and beef, and of time and dose on the reactive microcapsule trapping of benzo[*a*]pyrene metabolites in the rat gastrointestinal tract. Carcinogenesis 11, 599–607.

O'Neill, I.K., Povey, A.C., Bingham, S., Cardis, E., 1990b. Systematic modulation by human diet levels of dietary fibre and beef on metabolism and disposition of benzo[*a*]pyrene in the gastrointestinal tract of Fischer F344 rats. Carcinogenesis 11, 609–616.

Peters, R.K., Garabrant, D.H., Yu, M.C., Mack, T.M., 1989. A case-control study of occupational and dietary factors in colorectal cancer in young men by subsite. Cancer Research 49, 5459–5468.

Rigdon, R.H., Neal, J., 1966. Gastric carcinomas and pulmonary adenomas in mice fed benzo(a) pyrene. Texas Reports on Biology and Medicine 24, 195–207.

Rigdon, R.H., Neal, J., Mack, J., 1967. Leukemia in mice fed benzo(a)pyrene. Texas Reports on Biology and Medicine 25, 422–431.

Rigdon, R.H., Neal, J., 1969. Relationship of leukemia to lung and stomach tumors in mice fed benzo(a)pyrene. Proceedings of the Society for Experimental Biology and Medicine 130, 146–148.

Ross, J.A., Nelson, G.B., Wilson, K.H., Rabinowitz, J.R., Galati, A., Stoner, G.D., Nesnow, S., Mass, M.J., 1995. Adenomas induced by polycyclic aromatic hydrocarbons in strain A/J mouse lung correlate with time-integrated DNA adduct levels. Cancer Research 55, 1039–1044.

Santodonato, J., Howard, P., Basu, D., 1981. Health and ecological assessment of polynuclear aromatic hydrocarbons. Journal of Environmental Pathology and Toxicology 5, 1–376.

Schiffman, M.H., Felton, J.S., 1990. Re: "Fried foods and the risk of colon cancer". American Journal of Epidemiology 131, 376–378.

Soos, K., 1980. The occurrence of carcinogenic polycyclic hydrocarbons in foodstuffs in Hungary. Archives of Toxicology, Supplement 4, 446–448.

Sinha, R., Chow, W-H., Kuldorff, M., Denobile, J., Butler, J., Garcia-Closas, M., Weil, R., Hoover, R.N., Rothman, N., 1999. Well done, grilled red meat increases the risk of colorectal adenomas. Cancer Research 59, 4320–4324.

Sinha, R., Knize, M.G., Salmon, C.P., Brown, E.D., Rhodes, D., Felton, J.S., Levander, O.A., Rothman, N., 1998b. Heterocyclic amine content of pork products cooked by different methods and to varying degrees of doneness. Food and Chemical Toxicology 36, 289–297.

Sinha, R., Rothman, N., Brown, E.D., Salmon, C.P., Knize, M.G., Swanson, C.A., Rossi, S.C., Mark, S.D., Levander, O.A., Felton, J.S., 1995. High concentrations of the carcinogen 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP) occur in chicken but are dependent on the cooking method. Cancer Research 55, 4516–4519.

Sinha, R., Rothman, N., Salmon, C.P., Knize, M.G., Brown, E.D., Swanson, C.A., Rhodes, D., Rossi, S., Felton, J.S., Levander, O.A., 1998a. Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness and gravy made from meat drippings. Food and Chemical Toxicology 36, 279–287.

Steineck, G., Hagman, U., Gerhardsson, M., Norell, S.E., 1990. Vitamin A supplements, fried foods, fat and urothelial cancer. A case-referent study in Stockholm in 1985–87. International Journal of Cancer 45, 1006–1011.

Vaessen, H.A.M.G., Schuller, P.L., Jekel, A.A., Wilbers, A.M.M.A., 1984. Polycyclic aromatic hydrocarbons in selected foods: analysis and occurrence. Toxicological and Environmental Chemistry 7, 297–324.

Sugimura, T., Sato, S., Wakabayashi, K., 1988. Mutagens/carcinogens in pyrolysate of amino acids and proteins and in cooked foods: Heterocyclic aromatic amines. In: Woo, Y.T., Lai, D.Y., Arcos, J.C., Argue, M.F. (Eds.), Chemical Induction of Cancer, Structural Bases and Biological Mechanisms. Academic Press, New York, pp. 681–710.

Vaessen, H.A.M.G., Jekel, A.A., Wilbers, A.A.M.M., 1988. Dietary intake of polycyclic aromatic hydrocarbons. Toxicological and Environmental Chemistry 16, 281–294.

Ward, M.H., Sinha, R., Heineman, E.F., Rothman, N., Markin, R., Weisenburger, D.D., Correa, P., Zahm, S.H., 1997. Risk of adenocarcinoma of the stomach and esophagus with meat cooking method and doneness preference. International Journal of Cancer 71, 14–19.

Weyand, E.H., Chen, Y.-C., Wu, Y., Koganti, A., Dunsford, H.A., Rodriguez, L.V., 1995. Differences in the tumorigenic activity of a pure hydrocarbon and a complex mixture following ingestion: benzo[*a*]pyrene vs manufactured gas plant residue. Chemical Research in Toxicology 8, 949–954.

Weyand, E.H., Bevan, D.R., 1987. Species differences in disposition of benzo[*a*]pyrene. Drug Metabolism and Disposition 15, 442–448.

Yabiky, H.Y., Martins, M.S., Takahashi, M.Y., 1993. Levels of benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons in liquid smoke flavour and some smoked foods. Food Additives and Contaminants 10, 399–405.